For general laboratory use.
This product is not available in all territories due to different national regulations.
This document is not intended for use in the USA.

Print Protocol | Print Changes to Previous Version | Print Safety Precautions | Print License Disclaimer

DNA Process Control Kit

Version 03

Content version: October 2015

Includes DNA Process Control, Control Assay, and multiplex master mix for monitoring the whole workflow, from nucleic acid extraction to qPCR.

Cat. No. 07 339 542 001 1 kit
6 × 96 DNA Control reactions and 600 qPCR reactions of 20 µl final volume each

Cat. No. 07 339 666 001 1 kit
2 × 96 DNA Control reactions and 200 qPCR reactions of 20 µl final volume each

Refill Kit for the DNA Process Control detection only.

DNA Process Control Detection Kit

Cat. No. 07 339 623 001 1 kit
400 qPCR reactions of 20 µl final volume each

Store the kit at −15 to −25°C!

⚠ **Store protected from light!**

www.lifescience.roche.com

1. What this Product Does

Introduction

Two major outcomes can occur during a typical detection workflow for DNA targets. The sample can be either positive or negative. Whereas a positive detection also verifies the functionality of workflow components, a negative test outcome could be due either to a true negative sample or to a failure of critical workflow components and thus be a false negative. To rule out the possibility of workflow failures, a positive control that is similar to the target material but inert to the target detection is supplied in this kit and can be used in the workflow.

The DNA Process Control Kit contains a nuclease-resistant DNA that can be added at various steps during the nucleic acid purification of DNA detection workflows. Successful detection of this control DNA serves as a positive control, verifying the functionality of the DNA purification as well as the detection using quantitative PCR (qPCR). It also contains the LightCycler® Multiplex DNA Master optimized for multiplex reactions, ensuring performance for up to 4 targets plus the internal process control.

In order to prevent any cross-reaction with sample-derived nucleic acids or target-specific detection systems, the DNA Process Control Assay amplicon has been designed to have no significant homologies to any other known sequence. The DNA Process Control Detection Assay primers and probe are added in a low concentration to further lower any possible competition effects in multiplex PCR amplifications.

The DNA Process Control concentrate is formulated to achieve a robust Cq value within one specified workflow. Different sample materials and workflows may require adjusted dilution of the DNA Process Control concentrate.

The DNA Process Control Kit is designed for the detection of an endogenous heterologous control (DNA Process Control) during a sample purification procedure when using either the MagNA Pure 96 Instrument, MagNA Pure LC 2.0 Instrument, MagNA Pure Compact Instrument, or the High Pure Purification Kit.

The DNA Process Control Detection Kit (Cat. 07 339 623 001) includes the LightCycler® Multiplex DNA Master and the DNA Process Control Detection Assay. This is enough material for 400 detection reactions.

The DNA Process Control Detection Kit can only be used in combination with the DNA Process Control that is contained in the DNA Process Control Kit (Cat. No. 07 339 542 001 or 07 339 666 001).

Kit Contents

DNA Process Control Kit

Vial/ Bottle/Cap	Label	Function	Catalog Number
1 orange	DNA Process Control, conc.	Nuclease- resistant DNA concentrate in storage buffer	Cat. No. 07 339 542 001, 6 vials, 400 µl each Cat. No. 07 339 666 001, 2 vials, 400 µl each
2 white	DNA Process Control Diluent	DNA Process Control Diluent	Cat. No. 07 339 542 001, 3 bottles, 17 ml each Cat. No. 07 339 666 001, 1 bottle, 17 ml each
3 red	qPCR Reaction Mix (5×)	LightCycler® DNA Multiplex Master	Cat. No. 07 339 542 001, 3 vials, 880 µl each Cat. No. 07 339 666 001, 1 vials, 880 µl each
4 yellow	DNA Process Control Detection Assay (20×)	Primer/Probe Mix for detection of the DNA Process Control	Cat. No. 07 339 542 001, 6 vials, 120 µl each Cat. No. 07 339 666 001, 2 vials, 120 µl each
5 white	Water, PCR grade		Cat. No. 07 339 542 001, 12 vials, 1 ml each Cat. No. 07 339 666 001, 4 vials, 1 ml each

DNA Process Control Detection Kit

Vial/Bottle/ Cap	Label	Function	Catalog Number
3 red	qPCR Reaction Mix (5×)	LightCycler® DNA Multiplex Master	Cat. No. 07 339 623 001, 2 vials, 880 µl each
4 yellow	DNA Process Control Detection Assay (20×)	Primer/Probe Mix for detection of the DNA Process Control	Cat. No. 07 339 623 001, 4 vials, 120 µl each
5 white	Water, PCR grade		Cat. No. 07 339 623 001, 8 vials, 1 ml each

Storage and Stability

The kits are shipped on dry ice. Store the kits at −15 to −25°C in the dark.

The kit components are stable at −15 to −25°C until the expiration date printed on the label.

Store the DNA Process Control, conc. **(Vial 1)** at −15 to −25°C for up to 12 months.

- Avoid freeze thawing.

DNA Process Control working solution

- See Chapter **DNA Isolation**: How to prepare the working solution.
- Store the DNA process control working solution at +2 to +8°C for up to 1 week.

Store the DNA Process Control Diluent **(Vial 2)** at −15 to −25°C for up to 12 months.

- Store the DNA Process Control Diluent at +2 to + 8°C for a maximum of 4 weeks.

Store the qPCR Reaction Mix (5×) **(Vial 3)** at −15 to −25°C. Avoid repeated freeze/thaw cycles (more than 5×); aliquot Vial 3 and freeze or store Vial 3 at +2 to +8°C for a maximum of 4 weeks.

Store the DNA Process Control Detection Assay **(Vial 4)** at −15 to−25°C for up to 12 months.

- Avoid freeze thawing.
- See Chapter **Preparation of the master mix for automated PCR setup:** How to prepare the Detection Assay working solution.
- Store the Detection Assay working solution in the dark at +2 to +8°C for up to 1 week (optional for automated PCR setup workflows).

Store the Water, PCR grade **(Vial 5)** at +2 to +8°C for up to 12 months.

Although we recommend working on ice and preparing the qPCR reagents right before use, the Detection Assay working solution (everything combined except DNA template) is stable at +15 to +25°C up to 4 hours, and is therefore ideal for use in automated workflows.

Additional Equipment and Reagents Required

- Standard laboratory equipment
- Nuclease-free pipette tips
- 1.5 ml RNase/DNase-free microcentrifuge tubes to prepare master mixes and dilutions.
- To minimize risk of nuclease contamination, autoclave all vessels.
- Wear gloves at all times.

For qPCR:

- LightCycler® 480 Instrument II* or the LightCycler® 96 Instrument*
- LightCycler® 480 Multiwell Plate 96, white*
- LightCycler® 480 Multiwell Plate 384, white*
- Standard swing-bucket centrifuge with rotor for multiwell plates

For DNA Purification:

- MagNA Pure 96 Instrument* including disposables
- MagNA Pure 96 Internal Control Tube*, optional
- MagNA Pure 96 DNA and Viral NA Kit, Large Volume* or
- MagNA Pure 96 DNA and Viral NA Kit, Small Volume*

Alternatively, use other nucleic acid purification instruments and manual sample preparation products such as:

- MagNA Pure LC 2.0 Instrument* with MagNA Pure LC Total Nucleic Acid Isolation Kit*
- MagNA Pure Compact Instrument* with MagNA Pure Compact Nucleic Acid Isolation Kit I
- High Pure Viral Nucleic Acid Kit*

For Color Compensation with the LightCycler® 480 Instrument II:

- LightCycler® Multiplex DNA Master

Application

The DNA Process Control Kit is a tool to be used to control for potential failures of sample preparation, amplification, detection, and handling errors. The product is intended for use with a variety of sample materials (*e.g.*, blood, serum, stool, urine) and a variety of DNA targets.

The DNA Process Control Kit is intended for general laboratory use. Any use of the product for *in vitro* diagnostic tests is the sole responsibility of the operator and must be validated by the operator following the relevant national regulations.

Assay Time

The DNA Process Control purification step has various run times from 20 minutes (High Pure) up to 50 - 60 minutes for a typical run on the MagNA Pure 96 Instrument with 96 samples, depending on the protocol.
The DNA Process Control detection can be used with a fast qPCR protocol with run times of less than 60 minutes using the LightCycler® Multiplex DNA Master on the LightCycler® 480 Instrument II or LightCycler® 96 Instrument.

2. How to Use this Product

2.1 Before You Begin

Precautions

Use nuclease-free techniques. Nuclease-contaminated reagents and reaction vessels may degrade template DNA. Please follow these guidelines to minimize risk of contamination:

- Wear disposable gloves and change them frequently.
- Avoid touching surfaces or materials that could cause nuclease carry-over.
- Use only reagents provided in this kit. Substitutions may introduce nucleases.
- Clean and decontaminate work areas and instruments, including pipettes, with commercially available decontamination reagents.
- Use only new nuclease-free aerosol-blocking pipette tips.
- Use a work area specifically designated for nucleic acid work, and if possible use reaction vessels and pipettors dedicated only for work with template nucleic acid.

This product is for use by experienced personnel who have training in standardized molecular testing procedures and expertise in viral research, in laboratories with appropriate biosafety equipment and containment procedures.

Laboratory Procedures

All sample material and resulting waste should be considered potentially infectious. Thoroughly clean and disinfect all work surfaces with disinfectants recommended by the local authorities.

- Do not eat, drink, or smoke in the laboratory work area.
- Do not pipette by mouth.
- Wear protective disposable gloves, laboratory coats, and eye protection when handling specimens and kit reagents.
- Avoid microbial and nuclease contamination of reagents when removing aliquots from reagent bottles. Use sterile disposable pipette tips.
- Wash hands thoroughly after handling specimens and kit reagents.

In addition, to minimize the risk of carryover contamination which may result in false positive results, follow the guidelines listed below:

- Use a PCR hood.
- Wipe and UV-illuminate PCR workstations and biosafety cabinets before use.
- Have separate areas for sample preparation, PCR reaction setup, and PCR amplification.
- Discard pipette tips in sealed containers to prevent airborne contamination.
- The DNA Process Control concentrates and the working solution must be handled with care; open and prepare the solutions in a separate location.
- Avoid opening LightCycler® 480 Multiwell Plates containing amplification products.

Sample Material

Use any DNA suitable for PCR in terms of purity, concentration, and absence of PCR inhibitors. For reproducible isolation of nucleic acids, several options are possible.

For example:

- MagNA Pure 96 Instrument using the MagNA Pure 96 DNA and Viral NA Small Volume Kit, see Section 2.2.1.
- MagNA Pure 96 Instrument using the MagNA Pure 96 DNA and Viral NA Large Volume Kit, see Section 2.2.1.
- MagNA Pure LC Instrument using the MagNA Pure LC Total Nucleic Acid Kit in combination with the "Total NA HS 200" Protocol. Elution is performed using 100 µl elution buffer.
- MagNA Pure Compact Instrument using the MagNA Pure Compact Nucleic Acid Isolation Kit I in combination with the "Total_NA_Plasma_100_400" Protocol with 100 µl elution volume.
- High Pure Viral Nucleic Acid Kit (for manual isolation) with 200 µl specimen and 100 µl elution volume.

Options for Nucleic Acid Isolation and qPCR Using the DNA Process Control Kit

Nucleic Acid Isolation	qPCR
MagNA Pure 96 Instrument or MagNA Pure LC Instrument or MagNA Pure Compact Instrument or High Pure Viral Nucleic Acid Kit	LichtCycler® 480 Instrument II or LightCycler® 96 Instrument

Control Samples and No Template Controls (NTCs)

Always run appropriate negative and positive controls for each of your target parameters.

- In addition to the DNA Process Control, we highly recommend testing known negative and known positive specimens as controls in each run to check the complete procedure, including sample preparation and PCR.
- If the DNA Process Control is added manually to the sample material, we recommend having one known positive and one known negative specimen without the DNA Process Control.
- If the DNA Process Control is added automatically to all samples, we recommend performing a no template control (NTC) PCR using Water, PCR grade (Vial 5) instead of specimen eluate in the PCR. The negative control is necessary for determining potential contamination issues.

Primers

Suitable concentrations of PCR primers range from 0.2 to 0.5 µM (final concentration in PCR). The recommended starting concentration is 0.5 µM each.

Probes

Suitable concentrations of hydrolysis probes range from 0.05 to 0.5 µM (final concentration in PCR). The recommended starting concentration is 0.25 µM each.

- The optimal probe concentration is the lowest concentration that results in the lowest quantification cycle value (Cq) and adequate fluorescence dynamics for a given target concentration.
- To ensure efficient probe cleavage, the Tm of the hydrolysis probe has to be higher than the Tm of the primers.

2.2 Protocols

The DNA Process Control is added to the sample material directly or with the lysis buffer at the beginning of the procedure. Purification can be done using a variety of automated systems as indicated above. To control the workflow for a particular sample, the DNA Process Control can be used with target-specific assays using either a (one well) multicolor-multiplex format, or in two independent monoplex reactions.

For very low-copy targets, it is best to perform monoplex assays, as they generally produce higher sensitivity. In combination with appropriate positive target samples and valid NTCs, a positive DNA Process Control assay ensures correct sample processing during the nucleic acid purification and verifies the functionality of the qPCR detection reaction. The DNA Process Control assay is best used with the qPCR procedure described below.

The workflow for the MagNA Pure 96 nucleic acid purification and subsequent qPCR detection is described in detail in the following chapter.

In case of automated primary sample handling and automated PCR setup workflows, it can be beneficial to increase the pipetting volume of the individual reaction components. For this purpose, a 4× DNA Process Control Detection Assay working solution can be prepared.

2.2.1 Isolation of DNA

A) Preparation of the DNA Process Control working solution

The procedure below will result in a consistent DNA Process Control concentration that can be added to the sample material. The process control Cq values may vary depending on the type of sample material, the applied purification protocol, and workflow. It is recommended to use the protocol as outlined below and add 20 µl of the working solution to the sample material. If lower or higher Cqs from the DNA Process Control assay are required, the dilutions can be adjusted accordingly.

Step	Action
❶	Thaw one aliquot of the DNA Process Control, conc. (Vial 1) and one bottle of DNA Process Control Diluent (Bottle 2).
❷	Pipette 400 µl from Vial 1 and add to 3.6 ml DNA Process Control Diluent (Bottle 2). This will serve as the DNA Process Control working solution.
❸	Vortex briefly.

B) Example of a MagNA Pure 96 Instrument run using an Internal Control position

Step	Action
❶	Prepare the MagNA Pure 96 Instrument according to the Operator's Manual.
❷	Depending on the kit used, select one of the following protocols: • For large volume kit with 500 µl sample volume: • Pathogen Universal 500 • Viral NA Universal LV • Viral NA Plasma LV For small volume kit with 200 µl sample volume: • Pathogen Universal 200 • Viral NA Universal SV • Viral NA Plasma SV.
❸	Select elution volume: • 50 µl or 100 µl.
❹	Add the required volume of DNA Process Control working solution (A) to a MagNA Pure 96 Internal Control Tube and proceed according to the MagNA Pure 96 User Training Guide.

C) Other Nucleic Acid purification methods

- For the MagNA Pure LC, 20 µl of the DNA Process Control working solution is added directly into the sample material shortly before starting the purification process.
- For the MagNA Pure Compact, 20 µl of the DNA Process Control working solution is added directly into the sample material shortly before starting the purification process.
- For the High Pure Viral Nucleic Acid Kit, 20 µl of the DNA Process Control working solution is added directly into the sample material shortly before starting the purification process.

Follow the procedure below to prepare 96, 20 µl standard reactions with the LightCycler® DNA Multiplex Master, using either the LightCycler® 480 System or the LightCycler® 96 System and the LightCycler® 480 Multiwell Plate 96, white.

Do not touch the surface of the LightCycler® 480 Multiwell Plate 96 or 384.

2.2.2 Preparation of the qPCR

A1) Preparation of the master mix for manual qPCR setup

- Keep all reagents on ice.

Prepare master mix on ice as shown below for 95 samples plus 1 NTC (20 µl each, 10 µl amplifications included in brackets to be used for 384-well plate set-ups).

Vial	Component[1)]	Reagent Conc.	Master Mix for qPCR	
			1 Reaction	100 Reactions
3	qPCR Reaction Mix	5×	4 µl (2 µl)	400 µl (200 µl)
4	DNA Process Control Detection Assay	20×	1 µl (0.5 µl)	100 µl (50 µl)
×	Gene-Specific Primers		2 µl (1 µl)	200 µl (100 µl)
5	Water, PCR grade		8 µl (4 µl)	800 µl (400 µl)
	Master Mix Volume		**15 µl (7.5 µl)**	**1500 µl (750 µl)**

[1)] For eluates derived from stool samples, it is recommended to add 0.2 µg/µl (final) of molecular biology grade Bovine Serum Albumin. Adjust for BSA volume by subtracting from PCR Water volume.

A2) Preparation of the master mix for automated PCR setup

Preparation of the DNA Process Control Detection Assay working solution

For automated PCR setup environments that require larger pipetting volumes, such as the FLOW Solution, the DNA Process Control Detection Assay (20×) can be used to create a 4× working solution.

Step	Action
❶	Thaw one vial of the DNA Process Control Detection Assay, 20× (Vial 4) and one vial of Water, PCR grade (Vial 5).

Step	Action
❷	Add 480 µl water to Vial 4 (DNA Process Control Detection Assay, 20×) from Step 1.
❸	Vortex briefly.
❹	Add 5 µl of this 4× working solution to a 20 µl final qPCR reaction volume to achieve a final concentration of 1×.

- Keep all reagents on ice.

Prepare master mix on ice as shown below for 95 samples plus 1 NTC.

Vial	Component[1)]	Reagent Conc.	Master Mix for qPCR	
			1 Reaction	100 Reactions
3	qPCR Reaction Mix	5×	4 µl	400 µl
4	DNA Process Control Detection Assay	4×	5 µl	500 µl
×	Gene-Specific Primers		2 µl	200 µl
5	Water, PCR grade		4 µl	400 µl
	Master Mix Volume		**15 µl**	**1500 µl**

[1)] For eluates derived from stool samples, it is recommended to add 0.2 µg/µl (final) of molecular biology grade Bovine Serum Albumin. Adjust for BSA volume by subtracting from PCR Water volume.

B) Setting up the qPCR reaction

Step	Action
❶	Place your samples on ice. Prepare the qPCR master mix, as described in the pipetting protocol above (A1 or A2), and place on ice.
❷	• Dispense 15 µl of the reaction mixture qPCR master mix from A1 or A2 and the sample eluates to the respective wells of the pre-cooled LightCycler® 480 Multiwell Plate 96. Always start with the negative control, followed by the prepared specimen. Positive controls are pipetted last. • Add 5 µl Water, PCR grade (Vial 5) into the NTC position. • Add 5 µl sample eluate to all sample positions.
❸	Seal the LightCycler® 480 Multiwell Plate 96 with a LightCycler® 480 Sealing Foil.
❹	Place the multiwell plate in a standard swing-bucket centrifuge containing a rotor for multiwell plates with suitable adapters. Balance it with a suitable counterweight (*e.g.,* another multiwell plate), and centrifuge for 2 minutes at 1,500 × g.
❺	Proceed immediately with the qPCR. Avoid leaving the plate at +15 to +25°C.

2.2.3 LightCycler® 480 Instrument II Protocol

The DNA Process Control Kit is compatible with the LightCycler® 480 Instrument II, 96-well or 384-well.

The following procedure is optimized for use with the corresponding LightCycler® System you are using.

⚠ Program the LightCycler® Instrument before preparing the reaction mixes.

A LightCycler® Instrument protocol that uses the LightCycler® Multiplex DNA Master contains the following programs:

- **Denaturation of the DNA**
- **Amplification of the DNA**
- **Cooling of the thermal block**

For details on how to program the experimental protocol, see the current LightCycler® 480 Instrument Operator's Manual.

Programming a Customized Detection Format for the LightCycler® System Filter Combination Selection

The detection format in the LightCycler® 480 Instrument II Software, Version 1.5 setup needs to be customized for the applied dual-color or triple-color-hydrolysis format used in the qPCR detection

- Dual-color measurement and the simultaneous analysis of one additional parameter (*e.g.,* FAM, Yellow555, or Red610 channel), and the DNA Process Control (Cy5).
- Triple-color measurement and simultaneous analysis of multiple parameters (*e.g.,* FAM and Yellow555 or FAM and Red610) and the DNA Process Control (Cy5).

In the "Tool" module, the "Detection Formats" option allows creating new detection formats specified by the user, including a "Detection Format" list, a "Filter Combination" selection area, and "Selected Filter Combination List". Different filter settings for the LightCycler® 480 Instrument II are defined. Use the following filter combination:

Example of a (multi) 3-color Hydrolysis Probes filter combination:

Detection Formats	Excitation Filter	Emission Filter
FAM	465	510
Yellow555 or Red610	533 533	580 610
Cy5	618	660

For the new customized detection format, set the following values for all selected filters in the "Selected Filter Combination List":

Melting Factor	1
Quantification Factor	10
Integration Time	2

Protocol for use with the LightCycler® 480 Instrument II (Multiwell Plate 96 or 384)

Setup				
Detection Format			**Reaction Volume**	**Block Type**
For example: Detection format 3 color hydrolysis probe [FAM/Yellow555/Cy5]			20 µl (10 µl)	96 (384)
Programs				
Program Name			**Cycles**	**Analysis Mode**
Initial Denaturation			1	None
Amplification			45[1)]	Quantification
Cooling			1	None
Temperature Targets				
Target [°C]	**Acquisition Mode**	**Hold [hh:mm:ss]**	**Ramp Rate [°C/s]**	**Acquisitions [n/°C]**
Initial Denaturation				
95	None	00:00:30	4.4 (4.8)	–
Amplification				
95	None	00:00:05	4.4 (4.8)	–
60[2)]	Single	00:00:30	2.2 (2.5)	–
Cooling				
40	None	00:00:30	2.2 (2.5)	-

[1)] 45 cycles is suitable for most assays. If the assay shows steep amplification curves and early crossing points, 40 cycles should be sufficient. Reducing the number of cycles will improve uniformity of the product and reduce the time required for the assay.

[2)] The LightCycler® Multiplex DNA Master includes an aptamer for hot start. Anneal / Extend temperatures significantly below 60°C may not be well tolerated because aptamers will inhibit DNA polymerase activity at low temperatures.

Color Compensation Protocol for the LightCycler® 480 Instrument II

For a multicolor, multiplex amplification, the application of a color compensation file is necessary to compensate for optical crosstalk between the different channels.

For the LightCycler® 480 Instrument II, an instrument-specific color compensation file is mandatory for multicolor experiments, and a color compensation object can be generated by performing the following experiment.

The LightCycler® 480 Instrument II protocol contains the following programs:

- **Initial Denaturation** of the DNA
- **Amplification** of the DNA
- **Temperature Gradient Step** to create the Color Compensation file
- **Cooling** of the thermal block

For details on how to program the experimental protocol, see the LightCycler® 480 Software Operator's Manual, Version 1.5.

The following table shows the qPCR parameters that must be programmed for a LightCycler® 480 System Color Compensation file run with a LightCycler® 480 Multiwell Plate 96, white.

Setup			
Detection Format	**Block Type**		
Customized (see previous section)	96		
Programs			
Program Name	**Cycles**	**Analysis Mode**	
Initial Denaturation	1	None	
Amplification	45	Quantification	
Temperature Gradient Step	1	Color Compensation	
Cooling	1	None	
Temperature Targets			
Target [°C]	**Acquisition Mode**	**Hold [hh:mm:ss]**	**Ramp Rate [°C/s]**
Initial Denaturation			
95	None	00:00:30	4.4
Amplification			
95	None	00:00:05	4.4
60	Single	00:00:30	2.2
Temperature Gradient Step			
95	None	00:00:10	4.4
40	None	00:00:10	2.2
95	Continous		5 Acq. /°C
Cooling			
40	None	00:00:30	2.2

Preparation of the Color Compensation Run

Prepare the calibrator qPCR mix for more than one reaction; multiply the amount in the columns below by the number of reactions (minimum of 3 to 5 replicates). Note that there will be a slight loss of liquid during the pipetting steps. Please calculate extra volume of the qPCR mix by adding at least one additional reaction volume. In order to ensure accuracy, we do not recommend pipetting volumes below 1 µl when adding the individual reagents.

Component	1× Buffer	1× for Each Dye	1× DNA Process Control
qPCR Reaction Mix, 5× conc.(Vial 3)	4 µl	4 µl	4 µl
DNA Process Control Detection Assay, 20× (Vial 4)	-	-	1 µl
Detection mix for each dye (one dye per well)	-	X µl (depending on the assay)	
Water, PCR grade (Vial 2)	16 µl	Y µl (depending on the assay)	10 µl
Template, such as DNA or positive samples eluates	-	5 µl	-
DNA Process Control eluate	-	-	5 µl
Total Volume	**20 µl**	**20 µl**	**20 µl**

Step	Action
❶	Pipette the replicates of each different calibrator mix into a pre-cooled LightCycler® 480 Multiwell Plate 96.
❷	Seal the LightCycler® 480 Multiwell Plate using a sealing foil.
❸	Place the multiwell plate in a standard swing-bucket centrifuge containing a rotor for multiwell plates with suitable adaptors. Balance it with a suitable counterweight (*e.g.,* another plate), and centrifuge for 2 minutes at 1,500 × g.
❹	Load the multiwell plate into the LightCycler® 480 Instrument II and start the program.

Create Color Compensation Object

When the experiment is finished, open the **Sample Editor**. Select workflow **Color Comp** and designate the used positions of the Multiwell Plate as "Water" for Buffer replicates, and the appropriate dyes respectively (*e.g.*, FAM, Red610, Cy5 for the example mentioned above).

Open the **Analysis** module "Color Compensation", click on **Calculate**, and save the color compensation object in the database.

From this point on, you can apply this "CC Object" to each dual-, triple- or quadruple-color experiment performed with FAM, Yellow555, Red610, and Cy5 on the same instrument.

2.2.4 LightCycler® 96 Instrument Protocol

The following procedure is optimized for use with the LightCycler® 96 System. Program the LightCycler® 96 Instrument before preparing the reaction mixes. For details on how to program the experimental protocol, see the LightCycler® 96 Operator's Manual.

Run Editor			
Detection Format			**Reaction Volume**
Select Cy5 in Channel 4 and any other dye used in your assays.			20 µl
Programs			
Temp. [°C]	**Ramp Rate [°C/s]**	**Hold [s]**	**Acquisition Mode**
Preincubation (Initial Denaturation)			
95	4.4	30	None
2-Step Amplification			
No. of Cycles: 45[1)]			
95	4.4	5	None
60[2)]	2.2	30	Single

[1)] 45 cycles is suitable for most assays. If the assay shows steep amplification curves and early crossing points, 40 cycles should be sufficient. Reducing the number of cycles will improve uniformity of the product and reduce the time required for the assay.

[2)] The LightCycler® Multiplex DNA Master includes an aptamer for hot start. Anneal / Extend temperatures significantly below 60°C may not be well tolerated because aptamers will inhibit DNA polymerase activity at low temperatures.

Color Compensation Protocol for the LightCycler® 96 Instrument

The LightCycler® 96 Instrument does not require the creation of a color compensation object.

2.3 Quality Control

Each component of the three DNA Process Control Kits is function tested using the MagNA Pure 96 Instrument for the nucleic acid purification and the LightCycler® 480 Instrument II for qPCR.

3. Results

The DNA Process Control Kit was used according to the protocols described above. Plasma samples (180 µl) were processed on the MagNA Pure 96 Instrument with ± 20 µl of the DNA Process Control working solution. Heat-inactivated CMV virus was also processed on the MagNA Pure 96 Instrument, then amplified in either monoplex or duplex qPCR amplifications. As demonstrated below, Cqs generated from monoplex and duplex qPCR amplifications yielded very similar results, indicating that the presence and amplification of the DNA Process Control does not affect target amplification performance.

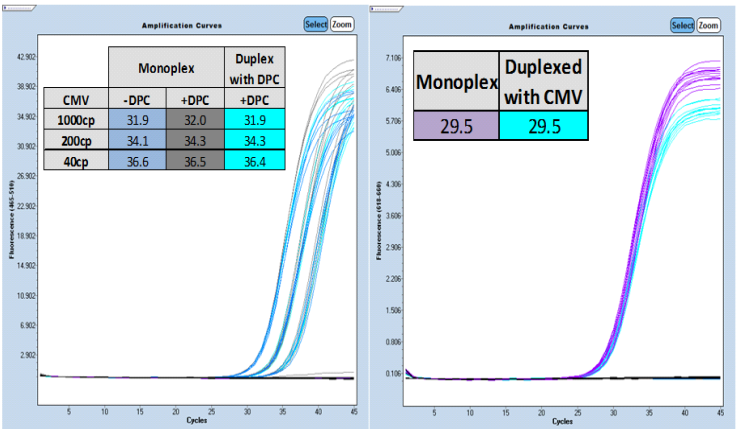

Fig. 1: Duplex / monoplex performance. Amplification curves of a CMV target and the DNA Process Control assay. The DNA Process Control (DPC) was added (±) to plasma samples and processed with the MagNA Pure 96 DNA and Viral NA Small Volume Kit using the Pathogen Universal 200 V3.0 protocol.

Usage with Different Sample Types

The DNA Process Control Kit was used in a panel of 5 different sample materials (EDTA-plasma, serum, whole blood, swab, and urine) according to the same standard protocol as described above (MagNA Pure 96 DNA and Viral NA Small Volume Kit using the Pathogen Universal 200 protocol with 100 µl eluate). Eluates were post-spiked with Human Reference cDNA and qPCR amplified in a duplex with the DNA Process Control.

All NTCs are clean and the DNA Process Control assay does not produce false positives in any of the sample materials.

The DNA Process Control assay performs comparably in all different sample types.

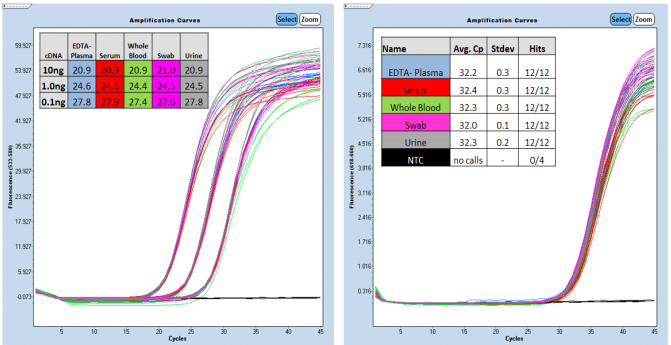

Fig. 2: Amplification curves of B2M2 target and the DNA Process Control (DPC) in various sample types. Twenty microliters DPC added to 180 ul of various sample types, then purified on the MagNA Pure 96 with the Viral NA Small Volume Kit using the Pathogen Universal 200 V3.0 protocol.

Interpretation of Data

The robust workflow described above ensures that a constant amount of DNA Process Control is added to the sample material. However, the detection of Cq values may vary depending on the overall workflow setup (used sample material, purification instrument and protocol, detection instrument, multiplexing parameters, researcher, etc.). In general, expect the Cq value to vary in a range of ~ +/- 2-3 Cqs for one particular setup.

Using the standard procedures with different nucleic acid purification protocols on different instruments with different sample materials, the DNA Process Control resulted in Cq values of ~30 - 35 (100 µl eluate and 5 µl eluate in a 20 µl qPCR detection reaction with the LightCycler® Multiplex DNA Master on a LightCycler® 480 Instrument II). For one such particular workflow, the following table shows an exemplary interpretation guideline.

Target (Channel 465 - 510)	DNA Process Control (Channel 618-660)	Results/ Target Status
no Cq	Valid Cq (~30 - 35)	Valid/ target negative
Cq < 40	Valid Cq or No Cq	Valid / target positive
no Cq	No valid Cq • For example, outside of ~30 - 35 • no sigmoid curve	Invalid/ target unknown

1. For any result assessments, do not solely rely on Cq callings. Ensure that the amplification curves are sigmoid in shape and that the Cq values are reasonable. The qPCR should be repeated if Cq values show an uncertain Character Code and Description of the status in the result screen (LightCycler® 480 Software, Version 1.5).
2. For a valid test batch (including negative controls with a "negative" result and positive controls with a "positive" result), verify each individual specimen for its result, including the DNA Process Control result.

Negative Control/ No Template Control (NTC)

The assay result for a negative control, measured in the appropriate channel, always needs to be negative. If the result is "positive", all specimen results controlled by the corresponding negative control are invalid because of potential contamination of the reagents. In the case of a "positive" result for the negative control, sample preparation and qPCR with the appropriate detector must be repeated.

Positive Controls

The assay result for the positive controls, measured in the appropriate channel, always needs to be positive. If the result is "negative", all specimen results controlled by the corresponding positive control are invalid, and the respective qPCR with the appropriate detector must be repeated.

Specimen Results

Check if the results of the negative and positive controls in the run are valid, and interpret the sample results for each target and DNA Process Control according to the table above (Interpretation of Data).

DNA Process Control / Internal Control

If specimen results are "negative" for a target, the simultaneous DNA Process Control measured in channel 618-660 always needs to be positive to prove that there actually was specimen material in the reaction and that the qPCR was not inhibited. For positive samples with a high amount of target DNA, the extraction control may be "negative" because of the competition of the two reactions.

4. Troubleshooting

	Possible Cause	Recommendation
Fluorescence intensity varies	Some of the reagent is still in the upper part of the microwell or an air bubble is trapped in the microwell.	Repeat centrifugation, but allow sufficient centrifugation time so that all reagent is at the bottom of the microwell and air bubbles are expelled.
	Skin oils or dirt on the surface of the microwell plate.	Always wear gloves when handling the multiwell plate.
Fluorescence intensity is very low	Low concentration or deterioration of dyes in the reaction mixtures because dye was not stored properly.	• Keep dye-labeled reagents away from light. • Store the reagents at −15 to −25°C and avoid repeated freezing and thawing.
	Poor PCR efficiency (reaction conditions not optimized)	• Check annealing temperature of primers and probes. • Check experimental protocol. • Optimize annealing temperature in the reverse transcription step or in the PCR reaction. • Always run a positive control along with your samples.
	DNA is degraded during isolation or improper storage.	• If possible, check DNA quality. • Check DNA with an established PCR primer when available.
	Pipetting errors and omitted reagents.	• Check for missing reagents. • Check the pipetting procedure.
	Impure sample material inhibits reaction.	• Dilute sample 1:10 and repeat the analysis. • Repurify the nucleic acids to ensure removal of inhibitory agents.
Negative control sample gives a positive signal	Contamination	Remake all critical reaction mixes. Use separate PCR setup working areas.

5. Additional Information on this Product

How this Product Works

The DNA Process Control Kit provides a non-competitive internal control to monitor nucleic acid purification and detection processes in order to prevent false-negative results. The DNA Process Control working solution contains a constant amount of nuclease-resistant DNA[1)] that can be added to a large variety of sample materials without impairing the purification of other sample intrinsic nucleic acid[2)], such as from DNA viruses.

The DNA Process Control Kit contains reagents for 6 × 96 control reactions and 600 reactions using the LightCycler® Multiplex DNA Master to detect the DNA targets in the eluates.

Test Principle

The control is added to the sample material and co-purified with all other sample endogenous nucleic acids. After purification, the DNA Process Control as well as any target-specific parameter is detected in a qPCR reaction.

- In monoplex reactions in single wells, the DNA Process Control must be positive.
- In multiplex reactions, the DNA Process Control detection must be positive in all target-negative samples. In target-positive samples, the DNA Process Control may be out-competed by the target-specific assay and a negative control result is allowed.

Any failure of the DNA Process Control Detection Assay in target-negative samples indicates an erroneous purification/detection workflow and the sample must be retested.

The key steps in the process are:

Step	Action
①	DNA Process Control working solution is added to the sample material.
②	The protective coat of the internal control is lysed during the extraction process, thus enabling co-purification of the control DNA with other sample endogenous nucleic acids.
③	The sample eluates are used for qPCR reactions • The DNA Process Control Detection Assay specifically detects the internal control. • Lab-developed assays assess the status of other targets in the sample material. Since the DNA Process Control is added to all samples, its successful detection proves the correct sample processing as well as the functionality of the generic detection reagents.

1) The strictly monitored production and quality processes in conjunction with the easy-to-use workflow ensure that constant amounts of the control DNA are added to each and every sample.

2) The DNA Process Control has a unique and completely artificial sequence that makes primer competition with any other target parameters less likely. The primer and the probe of the DNA Process Control are designed to only amplify and detect the unique sequence. The Cy5-labeled probe ensures that other target-specific assays with common dyes such as FAM, Y555, or Red610 can be combined in multiplex setups.

6. Supplementary Information

6.1 Conventions

6.1.1 Text Conventions

To make information consistent and easier to read, the following text conventions are used in this document:

Text Convention	Usage
Numbered instructions labeled ❶, ❷ etc.	Steps in a procedure that must be performed in the order listed.
Asterisk *	Denotes a product available from Roche Diagnostics

6.1.2 Symbols

In this document, the following symbols are used to highlight important information:

Symbol	Description
	Information Note: Additional information about the current topic or procedure.
⚠	Important Note: Information critical to the success of the procedure or use of the product.

6.2 Changes to Previous Version

- Information about the sample material 'template DNA derived from stool samples' has been added.
- Editorial Changes.

6.3 Ordering Information

Roche offers a large selection of reagents and systems for life science research. For a complete overview of related products and manuals, please visit and bookmark our homepage www.lifescience.roche.com:

- LightCycler® 480 System: www.lightcycler480.com
- LightCycler® 96 System: www.lightcycler96.com
- Automated Sample Preparation (MagNA Pure 96 System, MagNA Pure LC System and MagNA Pure Compact System): www.magnapure.com
- Manual Sample Preparation of Nucleic Acids: www.lifescience.roche.com/shop/en/us/products/manual-sample-preparation-of-nucleic-acids

	Product	Pack Size	Cat. No.
Instruments and Accessories	LightCycler® 480 Instrument II, 96 well	1 instrument (96 well)	05 015 278 001
	LightCycler® 480 Instrument II, 384 well	1 instrument (384 well)	05 015 243 001
	LightCycler® 480 Block Kit 96 Silver	1 block kit for 96-well PCR Multiwell Plates	05 015 219 001
	LightCycler® 480 Block Kit 384 Silver	1 block kit for 384-well PCR Multiwell Plates	05 015 197 001
	LightCycler® 480 Multiwell Plate 96, white	5 × 10 plates with sealing foils	04 729 692 001
	LightCycler® 480 Multiwell Plate 384, white	5 × 10 plates with sealing foils	04 729 749 001
	LightCycler® 480 Sealing Foil	50 foils	04 729 757 001
	LightCycler® 480 Software, Version 1.5	1 software package	04 994 884 001
	LightCycler® 96 Instrument	1 instrument	05 815 916 001
	MagNA Pure 96 Instrument	1 instrument plus accessories	06 541 089 001
	MagNA Pure 96 Internal Control Tube	150 tubes (15×10)	05 435 293 001
	MagNA Pure LC 2.0 Instrument	1 instrument plus accessories	05 197 686 001
	MagNA Pure Compact Instrument	1 instrument plus accessories	03 731 146 001
DNA Isolation Kits	MagNA Pure 96 DNA and Viral NA Small Volume Kit	3 sets for 192 isolations each	06 543 588 001

	Product	Pack Size	Cat. No.
	MagNA Pure 96 DNA and Viral NA Large Volume Kit	3 sets for 96 isolations each	06 374 891 001
	MagNA Pure LC Total Nucleic Acid Isolation Kit	1 kit (192 isolations)	03 038 505 001
	MagNA Pure Compact Nucleic Acid Isolation Kit I	1 kit for 32 isolations	03 730 964 001
	High Pure Viral Nucleic Acid Kit	1 kit for up to 100 purifications	11 858 874 001
Associated Kits	LightCycler® Multiplex DNA Master Kit	200 reactions (20 µl each) 1,000 reactions (20 µl each)	07 339 585 001 07 339 577 001
	Bovine Serum Albumin	1 ml 20 mg/ml	10 711 454 001

6.4 Disclaimer of License

NOTICE: For patent license limitations for individual products please refer to www.technical-support.roche.com.

6.5 Trademarks

LIGHTCYCLER, MAGNA PURE, and HIGH PURE are trademarks of Roche.

FAM is a trademark of Applera Corporation or its subsidiaries in the US and/or certain other countries.

All other product names and trademarks are the property of their respective owners.

6.6 Regulatory Disclaimer

For general laboratory use.

This product is not available in all territories due to different national regulations.

This document is not intended for use in the USA.

Contact and Support

If you have questions or experience problems with this or any Roche product for Life Science, please contact our Technical Support staff. Our scientists are committed to providing rapid and effective help.

Please also contact us if you have suggestions for enhancing Roche product performance or using our products in new or specialized ways. Such customer information has repeatedly proven invaluable to the research community worldwide.

To ask questions, solve problems, suggest enhancements or report new applications, please visit our Online Technical Support Site.

Visit lifescience.roche.com, to download or request copies of the following Materials:

- Instructions for Use
- Safety Data Sheets
- Certificates of Analysis
- Information Material

To call, write, fax, or email us, visit lifescience.roche.com and select your home country to display country-specific contact information.

1015.**07361572001**③

Roche Diagnostics GmbH
Sandhofer Strasse 116
68305 Mannheim
Germany